A STUDY OF THE ACTION OF NORDIHYDROGUAIARETIC ACID IN PREVENTING THE OXIDATION OF SOME COMPONENTS OF MILK FAT

BY

JOHN WARREN STULL

B.S., University of Illinois, 1942
M.S., University of Illinois, 1947

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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IN THE GRADUATE COLLEGE OF THE
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A STUDY OF THE ACTION OF NORDIHYDROGUAIARETIC ACID IN PREVENTING THE OXIDATION OF SOME COMPONENTS OF MILK FAT

The material in milk which is referred to as milk fat is important because of its monetary value and nutritional properties. In addition, milk fat contributes to the fine flavor, body, texture and appearance of dairy products.

The term milk fat as used herein is meant the complex aggregation of milk constituents which include the true fats or glycerides, naturally occurring free fatty acids, phospholipids, *beta*-carotene, vitamin A and traces of other fat soluble compounds such as tocopherol, cholesterol and ergosterol. Each of these constituents is subject to autoxidation. The oxidation of one or more of these components is responsible for the development of certain undesirable flavors which have been described as oxidized, tallowy, metallic, stale, cappy or cardboardy. Hereafter this flavor will be referred to as the oxidized flavor[1].

The rôle which each of the constituents of milk fat plays in the development of oxidized flavor is not entirely understood even though considerable progress has been made in studying various aspects of the problem. The situation is further complicated by the relationship between oxidative changes which occur in the milk fat and the influence of other milk constituents especially ascorbic acid.

In 1905 Golding and Feilmann (3) first reported on oxidized flavor in milk and described the defect as an "alkaline

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1. It should be pointed out that oxidative deterioration of milk fat may actually cause more than one type of off-flavor and for that reason the use of more than one term in the description of the off-flavors may be justifiable. In the other branches of the food industry, the term rancid is usually used synonymously with the term oxidized. In the dairy industry, oxidized is the term used to describe one type of off-flavor caused by oxidative changes in fat, whereas rancid is used to describe the flavor resulting from the hydrolytic splitting of fat.

mealy flavor". They traced the cause of the off-flavor to a cooper exposed milk cooler being used by the dairy encountering the difficulty. There followed an intense interest in the problems associated with the cause and prevention of oxidized flavor. Also, there was an attempt to determine which constituents of milk underwent oxidative deterioration during the production of oxidized flavor.

The glycerides are fairly stable to the oxidative influences commonly encountered in dairy products. This was pointed out in 1923 by Holm and Greenbank (4). They proposed that oleic acid oxidation was probably the phenoenon responsible for the prodution of oxidized flavor.

While studying the materials responsible for the richness of flavor of milk, Thurston and Barnhart (16) found that an oxidized flavor developed in a "synthetic milk" to which lecithin had been added. In the same year, Thurston *et al* (17) presented additional evidence that lecithin is the constituent in milk which is chemically changed in the development of oxidized flavor. As a result of this work it was shown that there was actually more than one type of off-flavor produced during the oxidation of milk fat. Thurston and his co-workers (17) described one flavor as oxidized and the other as tallowy. They showed that the tallowy flavor was produced from milk fat which had been washed free of lecithin.

In 1939, Josephson and Doan (6) confirmed the findings of Thurston and his co-workers (16,17) when they produced oxidized flavor by heating lecithin in distilled water in the presence of copper.

In 1941, Brown, Vanlandingham and Weakley (1) were unable to find a relationship between the carotene content of milk produced on dry feed and the intensity of metal-induced oxidized flavor. More recently, however, Krukovsky and co-workers (7,8,9,10) have shown a correlation between the development of tallowy or metallic flavor and the oxidation of carotene and vitamin A in milk fat.

Oxidized flavor may develop in dairy products even though

the factors which accelerate its development are controlled and the processing methods are carefully supervised in approved equipment. This fact has led to studies of the use of antioxidants in dairy products as a means of preventing or retarding the development of oxidized flavor. It has been found that one of the most effective antioxidants in dairy products is nordihydroguaiaretic acid (10,12,13,14,15). In this work a study has been made of some of the factors influencing the action of the antioxidant nordihydroguaiaretic acid (NDGA) in preventing or retarding the oxidation of some of the components of milk fat particularly the phospholipid fraction.

Experimental Procedure

The phospholpiid fraction used in this study was isolated by two methods. In the first method, the material was isolated from dried washed cream buttermilk which had been dried in a specially constructed lyophilization apparatus.

Near the end of the study, the supply of the phospholipid which had been isolated from lyophilized buttermilk became exhausted. In order to save time, the second procedure was adopted which involved isolating the phospholipid fraction from condensed washed cream buttermilk. By isolating the material from lyophilized buttermilk it was hoped to obtain an increased yield but there was no significant difference between the yield obtained in the two methods. Also, it required more than twice as much solvent in the extraction from washed cream buttermilk. An analysis of the composition of the phospholipid fraction isolated by the two methods shows that the two were comparable. The material isolated from the lyophilized buttermilk and the condensed buttermilk had iodine numbers of 35.64 and 35.86, 1.73 and 1.70 per cent nitrogen and 3.81 and 3.75 per cent phosphorus respectively. The phospholipid fraction was kept under an atmosphere of nitrogen at 4° C. in acetone until it was ready for use. When ready for use, the material was filtered with suction under a stream of nitrogen and the last traces of acetone were removed

in vacuo at 20° C.

In the preliminary experiments involving the use of washed milk fat, the fat was prepared by washing 8 times in a separatory funnel with 2 ml. water per gram of fat at a temperature of 40° C. It was then dried with anhydrous sodium sulfate.

The amount and rate of oxygen absorption was measured by using a Barcroft-Warburg respirometer. One gram of washed milk fat or 0.5 gram of the phospholipid fraction was used in the presence of 3.0 ml. of water which was used as a carrier for the various materials being studied. Fifteen ml. absorption flasks with one side arm were used. All measurements were made at 50° C. An atmosphere of oxygen was used as suggested by several investigators (2,5,11). By the use of a gassing manifold described by Umbreit *et al* (18), tank oxygen was allowed to flow through the flasks at a pressure of 8 pounds per square inch for 3 minutes. The flasks were then sealed immediately.

The presence and intensity of oxidized flavor was determined by combining the contents of the absorption flask with good flavored homogenized milk. In the preliminary experiments using phospholipid-free milk fat, the contents of the flask were tasted directly to detect the presence of oxidized flavor.

Experimental Results

The Oxygen Absorption of Washed Milk Fat

At the end of 480 minutes and in the presence of 2.0 and 4.0 p.p.m. added copper, the oxygen absorption values for the washed fat were 39.3 and 49.8 microlites per gram respectively. The fat did not have an oxidized flavor.

It was concluded that in order to be able to measure effectively the development of oxidized flavor as correlated with the oxygen absorption of washed milk fat it would be necessary to (a) use a larger absorption flask and

therewith a larger amount of material and/or (b) increase the temperature of the reaction to some point above 50° C. Provosions were not available for carrying out these two modifications of procedure so this phase of the study was not pursued further.

The Oxygen Absorption of the Phospholipid Fraction of Milk with and without Added Copper and NDGA

The results show that the optimum concentration of NDGA was from 0.001 to 0.01 per cent. Concentrations below 0.001 per cent were not high enough to be effective. Concentrations of 0.05 per cent and above accelerated oxygen absorption during the beginning of the oxidation reaction.

The Effect of Synergists on the Oxygen Absorption of the Phospholipid Fraction of Milk in the Presence of Added Copper and NDGA

Citric acid, ascorbic acid and methionine were found to have little or no antioxygenic action when used alone in the phospholipid fraction containing added copper. In the presence of NDGA, they were found to be effective synergists. Citric acid and ascorbic acid were equally effective while there was an indication that methionine was slightly less effective.

The Effect of Metallic Catalysts on the Oxygen Absorption of the Phospholipid Fraction of Milk with and without NDGA

The effect of the metallic catalysts copper, nickel, iron and cobalt were studied. There was a slight but definite difference in the catalytic effectiveness of these metals both with and without NDGA. In the order of greatest effectiveness they were copper, nickel, iron and cobalt.

The Oxygen Absorption of the Phospholipid Fraction of Milk in the Presence of NDGA as Influenced by pH

It was shown that NDGA was most effective at pH 6.5 while it was less effective at pH 7.0, 7.5 and 8.0. NDGA

showed very little antioxygenic action at pH 3.0. The effectiveness was increased greatly as the pH was raised from 3.0 to 4.0, 5.0 to 6.0.

The Oxygen Absorption of the Phospholipid Fraction of Milk as Influenced by the Addition of NDGA alone and together with Citric Acid at the End of 1,2,3,5 and 7 Hours of the Oxygen Absorption Reaction

The antioxidant alone and together with citric acid was introduced from the side arm of the absorption flask at the end of 1,2,3,5 and 7 hours of the reaction. It was shown that NDGA alone or with citric acid would retard oxidation which had been allowed to proceed for a period of time of from 1 to 7 hours.

Literature Cited

(1) Brown, W. C., Vandingham, A. H. and Weakley, C. E. Oxidized Flavor in Milk. IX. The Effect of the Quality of Hay and Early Stage of Lactation on the Carotene Content of Butterfat and on the Ascorbic Acid Content of the Milk and their Relationship to the Development of Metal-induced Oxidized Flavor. J. Dairy Sci., 24: 925-936. 1941.

(2) Elder, L. Staling vs Rancidity in Roasted Coffee. Oxygen Absorption of the Fat Fraction. Ind. and Eng. Chem., 29: 267-270. 1937.

(3) Golding, J. and Feilman, E. Taint in Milk due to Contamination by Copper. Soc. Chem. Indus. J., 24: 1285-1286. 1905.

(4) Holm, G. E. and Greenbank, G. R. Quantitative Aspects of the Kreis Test. Ind. Eng. Chem., 15: 1051-1053. 1923.

(5) Johnston, W. R. and Frey, C. N. Autoxidation Measurements on Fatty Oils Using Barcroft-Warburg Apparatus. Ind. Eng. Chem., Anal. Ed., 13: 479-481. 1941.

(6) Josephson, D. V. and Doan, F. J. Observations on Cooked Flavor in Milk — its Source and Significance. Milk Dealer, **29**: 35-37, 54-62. 1939.

(7) Krukovsky, V. N., Ellis, G. H., and Percy, W. R. The Oxidation of Vitamin A and Carotene in Milk Fat. J. Dairy Sci., **26**: 869-875. 1943.

(8) Krukovsky, V. N., Ellis, G. H., and Barnes, B. W. Further Studies on Oxidation of Vitamin A and Carotene in Milk Fat. J. Dairy Sci., **27**: 249-255. 1944.

(9) Krukovsky, V. N., Guthrie, E. S., and Whiting, Frank. Lipid Deterioration in Dairy Products. The Stability of Milk Fat and the Fat-soluble Vitamins as Determined by the Re-emulsification Test. J. Dairy Sci., **31**: 961-973. 1948.

(10) Krukovsky, V. N., Theokas, D. A., Whiting, Frank, and Guthrie, E. S. The Effects of Nordihydroguaiaretic Acid, Salt and Temperature of Storage on the Stability of Fat and Fat-soluble Vitamins in Cream and Butter. J. of Dairy Sci., **32**: 679-688. 1949.

(11) Myers, J. E., Kass, J. P., and Burr, G. O. Comparative Rates of Oxidation of Isomeric Linolenic Acids and their Esters. Oil and Soap, **18**: 107-109. 1941.

(12) Richardson, G. A., and Long, Louisa, M. The Use of Carotene for Coloring Butter. J. Dairy Sci., **30**: 533-534. 1947.

(13) Stull, J. W., Herreid, E. O., and Tracy, P. H. A Study of the Use of Nordihydroguaiaretic Acid in Dairy Products. I. Its Antioxygenic Properties in Milk. J. Dairy Sci., **31**: 449-454. 1948.

(14) Stull, J. W., Herreid, E. O., and Tracy, P. H. A Study of the Use of Nordihydroguaiaretic Acid in Dairy Products. II. Its Antioxygenic Properties in Unsweetened Frozen Cream. J. Dairy Sci., **31**: 1024-1028. 1948.

(15) Stull, J. W., Herreid, E. O., and Tracy, P. H. A

Study of the Use of the Nordihydroguaiaretic Acid in Dairy Products. III. Its Antioxygenic Properties in Sweetened Frozen Cream. J. Dairy Sci., 32: 301-305. 1949.

(16) Thurston, L. M., and Barnhart. A Study of the Relation of Materials Adsorbed on the Fat Globules to the Richness of Flavor of Milk and Certain Milk Products. J. Dairy Sci., 18: 131-137. 1935.

(17) Thurston, L. M., Brown, W. C., and Dustman, R. B. Oxidized Flavor in Milk. I. The Probable Relation of Lecithin to Oxidized Flavor. J. Dairy Sci., 18: 301-305. 1935.

(18) Umbreit, W. W., Burris, R. H., and Stauffer, J. F. Manometric Techniques and Related Methods for the Study of Tissue Metabolism. 2nd ed., Burgess Publishing Co., Minneapolis, Minnesota. 1949.

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Stull, J. W., Herreid, E. O. and Tracy, P. H. A Study of the Use of the Antioxidant Nordihydroguaiaretic Acid in Dairy Products. I. Its Antioxygenic Properties in Milk. Dairy Sci., **31**: 449-454. 1948.

Stull, J. W., Herreid, E. O. and Tracy, P. H. A Study of the Use of the Antioxidant Nordihydroguaiaretic Acid in Dairy Products. II. Its Antioxygenic Properties in Unsweetened Frozen Cream. Dairy Sci., **31**: 1024-1028. 1948.

Stull, J. W., Herreid, E. O. and Tracy, P. H. A Study of the Use of the Antioxidant Nordihydroguaiaretic Acid in Dairy Products. III. Its Antioxygenic Properties in Sweetened Frozen Cream. Dairy Sci., **32**: 301-305. 1949.

Stull, J. W. and Herreid, E. O. A New Lyophilizing Apparatus. Dairy Sci., **32**: 973-977. 1949.

Stull, J. W. Storage of Cream for Use in Ice Cream. Ice Cream Trade J., **46**: 46,86. 1950.